IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	Hudson Freeze *et al.*		
Serial No.:	10/500,480	Group No.:	1651
Filed:	12/13/2004	Examiner:	MacAuley, S.R.
Entitled:	**Novel Ligand Involved In the Transmigration of Leukocytes**		

DECLARATION UNDER 37 C.F.R. §1.132 BY DR. GEETHA SRIKRISHNA

Mail Stop AMENDMENT
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Examiner MacAuley:

1. I, the undersigned, Geetha Srikrishna, am a co-inventor of the above-referenced application, and am Research Assistant Professor at Sanford-Burnham Medical Research Institute, 10905 Road to the Cure, San Diego, CA 92121. My *Curriculum Vitae* is enclosed (Tab A).

2. I am co-author of Srikrishna *et al.* "A novel anionic modification of N-Glycans on mammalian endothelial cells is recognized by activated neutrophils and modulates acute inflammatory responses," (2001) The Journal of Immunology, January 1, 2001, vol. 166 no. 1 624-632 (Tab B).

3. Srikrishna *et al.* teaches a zymosan-induced animal model of acute peritoneal inflammation, in which "peritonitis was induced by intraperitoneal injection of 1 mg of zymosan in 0.5 ml PBS."[1] This model was used to demonstrate that

> "Intravenous injection of mAb GB3.1 immediately before the induction of peritonitis resulted in a dose dependent reduction in the extent of neutrophil and monocyte accumulation, while an isotype-matched mouse IgG or mAbAD7.5 or an unrelated anti-carbohydrate Ab (9) had minimal effect (Fig. 9)."[2]

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1 Srikrishna *et al.*, page 626, 1st column, 6th and 7th paragraphs.
2 Srikrishna *et al.*, page 630, 2nd column, 1st paragraph; and Figure 9.

4. The Specification of the instant patent application teaches methods for using an art-accepted *in vivo* animal model of inflammation for screening, using routine experimentation. In particular, the Specification's Example 10 teaches a zymosan-induced animal model of acute peritoneal inflammation,[3] in which "peritonitis was induced by intraperitoneal injection of 1 mg of zymosan in 0.5 ml PBS."[4] The Specification further teaches in Example 34 the successful use of antibodies to reduce inflammation in the *in vivo* animal model.[5]

The Specification's *in vivo* animal model is the same as the *in vivo* animal model of inflammation that was published in the above-discussed peer reviewed article.

Based on the above-discussed teachings by each of Srikrishna *et al.* and the Specification regarding the use of an animal model of inflammation, it is my opinion that these teachings may be applied by one of ordinary skill in the art, using routine experimentation, to screen test agents for their effect on inflammation *in vivo*.

5. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing therefrom.

Dated: 09/23/2011 Signed:

Dr. Geetha Srikrishna

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3 Specification, Example 10, pages 96-99.
4 Specification, page 97, lines 4-5.
5 Specification, Example 34, pages 129-131.

CURRICULUM VITAE
GEETHA K. SRIKRISHNA

Business Address	Genetic Diseases Program Sanford-Burnham Medical Research Institute 10901 North Torrey Pines Road La Jolla, California 92037
Phone	858-795-5256
E-mail	gsrikrishna@sanfordburnham.org
Current position	Research Assistant Professor

EDUCATION

1983-1988: **Doctor of Philosophy**. Christian Medical College and Hospital, Vellore, India, affliated to the University of Madras, India; degree awarded under the Faculty of Medicine. Thesis work was done in concordance with a clinical position in the Department of Clinical Biochemistry as listed below. Concentration areas: Placental function and Reproductive Endocrinology. Thesis: "Cystine aminopeptidase (oxytocinase) enzyme systems of primate placental and non-placental tissues". External Reviewers of thesis: Dr. Richard H. Gadsden, Professor of Pathology and Laboratory Medicine, Medical University of South Carolina, Charleston, SC and Dr. T.N. Pattabiraman, Professor of Biochemistry, Kasturba Medical College, Manipal, India.

1976-1979: **Master of Science**, Christian Medical College, Vellore, affiliated to the University of Madras, India. **Major**: Biochemistry with specialization in Clinical Biochemistry. Degree awarded under Faculty of Medicine for extended three-year course studies and laboratory training in Human Anatomy, and Physiology, and training and internship in the Clinical Laboratory of Christian Medical College Hospital, Vellore, India.

1972-1975: Bachelor of Science, Auxilium College, Vellore, affiliated to the University of Madras, India. **Major**: Chemistry, **Minor:** Physics and Biology

POSTDOCTORAL STUDIES:

1988-1993: Christian Medical College, Vellore, India in concordance with an academic position as listed below. Concentration: Neuroendocrinology.

1994-2001: Burnham Institute, La Jolla, California. Concentration: Glycobiology.

PROFESSIONAL APPOINTMENTS

2006 to present:	Research Assistant Professor, Sanford-Burnham Medical Research Institute, La Jolla, California
2002 to 2006:	Staff Scientist, The Burnham Institute for Medical Research, La Jolla, California
1991-1994	Lecturer (Academic Appointment) Dept of Clinical Biochemistry, Christian Medical College and Hospital, Vellore, India,
1988-1991	Associate Clinical Biochemist Grade I (Academic Appointment) Dept of Clinical Biochemistry, Christian Medical College and Hospital, Vellore, India,
1979-1987	Associate Clinical Biochemist Grade IV through Grade II (Clinical Appointments) Dept of Clinical Biochemistry, Christian Medical College and Hospital, Vellore, India

RESEARCH AT THE SANFORD-BURNHAM MEDICAL RESEARCH INSTITUTE

Lead Project: Studying the role of damage-associated molecular pattern (DAMP) molecules and their carbohydrate recognition epitopes in mediating mucosal inflammation and cancers. Work done through many years led to several discoveries: Novel anionic glycans, referred to as carboxylated glycans, show restricted expression on human and mouse cells of the myeloid lineage, and bind DAMP molecules HMGB1 and S100 proteins. S100A8 and S100A9, well-known pro-inflammatory molecules, have multiple roles in promoting malignancy as secretory products in the tumor microenvironment. This includes promotion of autocrine accumulation of myeloid-derived suppressor cells (MDSC) in tumors, and activating signaling pathways in tumor cells leading to expression of several pro-tumorigenic gene products. An antibody generated against carboxylated-glycans, expressed on RAGE and other cell surface glycoproteins, blocks the onset of colitis and colitis associated colon carcinogenesis in mice, and accumulation of MDSC in a 4T1 model of metastatic mammary tumor.

RESEARCH SUPPORT

Ongoing Research Support

P30 CA030199-30 Pilot Project Srikrishna (PI) 05/01/11 04/30/12
NIH/NCI/Sanford-Burnham Cancer Center
Myeloid-derived Suppressor Cells in B-cell lymphoma
In collaboration with the Rickert lab, this study seeks to investigate the role of myeloid-derived suppressor cells in the progression of B-cell lymphoma. **Role: Principal Investigator**

R21 CA127780 Srikrishna (PI) 01/01/2009-12/31/2011

(No cost extension)
NIH/National Cancer Institute
S100A8/A9 and carboxylated N-glycans in inflammation-mediated colon cancer
This study seeks to define the role of S100A8/A9 and carboxylated glycans in colitis mediated colon carcinogenesis using mouse models of disease. **Role: Principal Investigator**

A1 submission due

R21 CA161860-01 Srikrishna (PI) 07/01/2011-06/30/2013
NIH/National Cancer Institute
S100A8/A9 induced proteins as colon cancer biomarkers
This study seeks to measure S100A8/A9 induced proteins in tissues and serum of two large cohorts of colorectal cancer patients to develop a prognostic biomarker panel that would identify early stage patients at high risk of disease recurrence. **Role: Principal Investigator**

Completed Research Support

Crohn's and Colitis Foundation of America Freeze (PI) 01/02/07-12/31/08
Molecular basis of a novel therapeutic antibody for IBD
This study sought to identify relevant cell types and the role of RAGE in the blocking effects of the anti-glycan antibody in colitis. **Role: Co-investigator**

R01 CA092608 Freeze (PI) 7/1/02 - 6/30/07
NIH/National Cancer Institute
Novel Carboxylated Glycans in Cell Adhesion
This proposal studied the structure of novel carboxylated glycans on Receptor for Advanced Glycation End products (RAGE), determine their contribution to ligand binding, and assess the roles they play in mediating HMGB-1-RAGE interactions in tumor growth and metastasis. **Role: Co-investigator**

5 R21 GM065323-02 Freeze (PI) 04/01/02- 03/31/05
NIH/NIGMS
Novel Carboxylated N-Glycans that Mediate Inflammation
The major goal of this project was to establish the structure of these novel glycans. **Role: Co-investigator**

BMRP IBD-0021 Freeze (PI) 8/1/02 – 3/31/05
Eli and Edythe L. Broad Foundation
This study investigated the efficacy of the Anti-Glycan Antibody (mAbGB3.1) as a Novel Therapy for IBD.
Role: Co-investigator

PROFESSIONAL MEMBERSHIP

Society for Glycobiology
American Association for Cancer Research

PATENTS

20100249383: Novel ligand involved in the transmigration of leukocytes across the endothelium and uses therefore

20100021472: Methods for diagnosing and treating cancer

OTHER PROFESSIONAL EXPERIENCE

REVIEWERSHIP

Manuscript Review: Immunology, Clinical and Experimental Immunology, Leukemia Research, Journal of Biological Chemistry, Journal of Leukocyte Biology, Virology, Clinical and Experimental Metastasis

Grant Review: Wellcome Trust, UK
Wellcome Trust- Dept of Biotechnology India Alliance
FWO-Research Foundation, Flanders Belgium

SANFORD-BURNHAM COMMUNITY OUTREACH

Member, Community Task Force
Member, Rewards and Recognition Committee

SAN DIEGO COMMUNITY OUTREACH

Volunteer 2006-present: The Preuss School, UCSD. Tutor, Saturday Enrichment Academy, Review of Applications, Senior presentations, Science Fair presentations.

2001-2004: Volunteer, Greater San Diego Science and Engineering Fair
2001-2004: Volunteer, San Diego Humane Society

TEACHING EXPERIENCE

1979-1993 Christian Medical College, Vellore, India

Classroom/laboratory courses, approximately 4 hrs/week

- Lectures and laboratory course classes in lipid metabolism, immunology, endocrinology and organ function tests to medical and allied health undergraduate students.
- Course-developing for clinical biochemistry and clinical pathology technical diploma students.
- Thesis advisor for clinical residents.
- Lectures for graduate students in biochemistry, and clinical residents in medicine and pathology.

LABORATORY MEDICINE EXPERTISE

1979-1993 Christian Medical College, Vellore, India

Involved in laboratory medicine for 14 years in the Dept of Clinical Chemistry, a WHO Regional Reference laboratory of Christian Medical College Hospital, a 2000-bed internationally renowned tertiary medical center.

Responsibilities (approximately 40 hrs/week)

- Handling specialized diagnostic work-up
- Implementing new diagnostic assay methods
- Participation in clinical meetings
- Interaction with physicians on a regular basis to discuss clinical diagnosis and prognosis, especially of rare clinical disorders
- Inspecting all out-going patient results from the laboratory on a twice-daily basis and quality control monitoring
- Administrative responsibilities included training, overseeing and scheduling the work of the technical staff of the Dept of Clinical Biochemistry (more than 40 personnel).

HONORS AND AWARDS

- Invited talks/oral presentations: International Symposium on Molecular Cell Biology of Macrophages, Kumamoto, Japan; Eli Lilly laboratories, Indianapolis 2003; Keystone meeting on Mucosal inflammation 2003; Annual Glycobiology meeting, Boston 2000 etc.
- Best presentation, La Jolla Immunology Conference, 2003
- AV Tilak Parvathi Prize 1993 for outstanding neurological studies in bipolar disorders, national award by the Neurological Society of India
- Independent Research funding (1984-1987) for graduate studies on "Oxytocinases in health and disease" from the Council of Scientific and Industrial Research, New Delhi, India
- First Rank in Bachelor of Science, Chemistry Major, University of Madras, 1975
- CK Sundaram Iyer Prize for outstanding performance in BS Chemistry major, University of Madras, 1975
- Best Outgoing student of the undergraduate class of 1975, Auxilium College, Vellore, India
- National Merit Scholarship from the Government of India, supporting undergraduate and graduate studies

CONFERENCES WHERE WORK WAS PRESENTED

2010 International Symposium on Molecular Cell Biology of Macrophages, Kumamoto, Japan
2009 Society of Glycobiology Annual Meeting, San Diego, CA
2009 Regulatory Myeloid Suppressor Cells in Health and Disease, Clearwater Beach, FL
2008 AACR meeting on Inflammation and Cancer, Ko Olina, Oahu, HI
2008 Keystone meeting on Inflammation, Microenvironment and Cancer, Snowbird, UT
2008 La Jolla Immunology Conference, La Jolla, CA

2007 Keystone Meeting on Mechanisms linking Inflammation and Cancer, Santa Fe, NM
2005 Keystone Meeting on Inflammation and Cancer, Breckenridge, CO
2005 Broad Medical Research Program Annual Meeting, Los Angeles, CA
2003 Annual Meeting of the Society of Glycobiology, San Diego, CA
2003 La Jolla Immunology Conference, La Jolla, CA
2003 Keystone Meeting on Mucosal Inflammation, Keystone, CO.
2002 Annual Meeting of the Society of Glycobiology, Boston MA
2002 Keystone Symposium of Inflammatory Paradigms and the Vasculature, Steamboat Springs, CO
2001 Annual meeting of the Society of Glycobiology, San Francisco, CA
2001 Arthritis Research Conference, San Diego, CA
2000 Annual meeting of the Society of Glycobiology, Boston, MA
1998-2010 San Diego Glycobiology Symposia
1999 Keystone Symposium of Inflammatory Paradigms and the Vasculature, Santa Fe, NM
1997 Gordon Research Conference on Glycobiology, Ventura, CA
1985-1992 Annual Meetings of the Association of Clinical Biochemists of India
1987 Xth World Congress of IUPHAR (International Union of Pharmacology), Sydney, Australia
1985-1988 Annual Meetings of the Society of Biological Chemists of India

PUBLICATIONS

Most relevant to the lead project at SBMRI (senior/corresponding authorship underlined).

1. **Srikrishna, G**., Toomre, D.K., Manzi, A., Panneerselvam, K., Freeze, H.H., Varki, A., & Varki, N.M. (2001) A novel anionic modification of N-glycans on mammalian endothelial cells is recognized by activated neutrophils and modulate acute inflammatory responses. ***Journal of Immunology*** 166:624-632.
2. **Srikrishna, G**., Panneerselvam, K., Westphal, V., Abraham, V., Varki, A., & Freeze, H.H (2001) Two proteins modulating transendothelial migration of leukocytes recognize novel carboxylated glycans on endothelial cells. ***Journal of Immunology*** 166:4678-4688.
3. **Srikrishna, G.,** Huttunen, H., Johansson, L., Weigle, B., Yamaguchi, Y., Rauvala, H., & Freeze, H.H (2002) Novel N-glycans on the Receptor for Advanced Glycation End products (RAGE) influence amphoterin binding and neurite outgrowth. ***Journal of Neurochemistry*** 80:998-1008
4. **Srikrishna, G.**, Brive, L., Freeze HH. Novel carboxylated glycans contain oligosaccharide-linked glutamic acid (2005). ***Biochem Biophys Res Commun***. 332, 1020-7.
5. **Srikrishna, G.** Turovskaya, O., Shaikh R., Newlin R., Foell, D., Murch, S., Kronenberg M and Freeze H.H.(2005) Carboxylated glycans mediate colitis through activation of NF kappa B. ***Journal of Immunology***. 175, 5412-22. (article received editorial commentary)
6. Turovskaya, O, Foell, D, Sinha, P, Vogl T, Newlin, R., Nayak, J., Nguyen, M., Nawroth, P., Bierhaus, A., Varki, N., Kronenberg, M., Freeze, H and **Srikrishna, G** (2008). RAGE, carboxylated glycans and S100A8/A9 play essential roles in colitis-associated carcinogenesis. ***Carcinogenesis***, 29, 2035-43. PMCID: PMC2556970
7. Sinha, P., Okoro, C., Foell, D., Freeze H., Ostrand-Rosenberg, S and **Srikrishna, G.** (2008). Proinflammatory proteins regulate the accumulation of myeloid derived suppressor cells. ***Journal of Immunology,*** 181, 4666-75. (Article featured under Research Highlights in Nature Reviews Immunology Nov 2008 and in SciBx, a Nature/Biocentury venture publication). PMCID: PMC2810501

8. van Lent, P., Grevers, L, Blom, AB, Arnzt, O., van de Loo, FAJ., van der Kraan P, Abdollahi-Roodsaz, **Srikrishna, G**, Freeze, H, Sloetjes, A, Nacken W, Vogl T., Roth, J and van den Berg, WB (2008). S100A8 stimulates chondrocyte mediated cartilage destruction during experimental murine arthritis. ***Arthritis Rheum.*** *58(12): 3776-87.*
9. **Srikrishna, G** and Hudson H. Freeze (2009). Endogenous Damage Associated Molecular Pattern (DAMP) molecules at the crossroads of inflammation and cancer. ***Neoplasia***, Review Article (2009) 11: 615-28. PMCID: PMC2697348
10. **Srikrishna, G**, Nayak, J., Weigle, B., Temme, A., Foell, D., Hazelwood, L., Olsson, A., Volkmann, N., Hanein, D., and Freeze, H. (2010). Carboxylated N-glycans on RAGE promote S100A12 binding and signaling. ***Journal of Cellular Biochemistry***. 110:645-59. PMCID: PMC2879712
11. Ichikawa, M, Williams, R, Wang, L, Vogl, T and **Srikrishna, G**. 2011. S100A8/A9 activate key genes and pathways in colon tumor progression. ***Molecular Cancer Research*** 9:133-148 (article featured in highlights of the issue). PMCID: PMC3078037.
12. **Srikrishna, G.** 2011. S100A8 and S100A9. New Insights into their roles in malignancy (review). ***Journal of Innate Immunity*** (accepted).

Other publications in glycobiology from the Freeze Lab, Sanford-Burnham Medical Research Institute

1. Etchison, J., **Srikrishna, G** and Freeze, H (1995). A novel method to co-localize glycoaminoglycan core oligosaccharyl transferases in the rat liver Golgi. *Journal of Biological Chemistry*, 270: 756-764. PMID:7822307
2. **Srikrishna, G.**, Varki, N.M., Newell, P.C., Varki, A., & Freeze, H.H. (1997) An IgG monoclonal antibody against *Dictyostelium discoideum* glycoproteins specifically recognizes Fucb1, 6 GlcNAcb in the core of N-linked glycans. *Journal of Biological Chemistry*, 272:25743-25752. PMID: 9325301
3. **Srikrishna, G.**, Wang, L., & Freeze, H.H. (1998) Fucoseb1-P-Ser is a new type of glycosylation: Using antibodies to identify a novel structure in Dictyostelium discoideum and study multiple types of fucosylation during growth and development. ***Glycobiology***, 8:799-811 PMID:9639541
4. Marquardt, T., Luhn, K., **Srikrishna, G.**, Freeze, H.H., Harms, E., & Vestweber, D. (1999) Correction of leukocyte adhesion deficiency type II with oral fucose. *Blood*, 94:3976-3985. PMID;10590041
5. Kim, S., Westphal, V., **Srikrishna, G.**, Mehta, D.P., Peterson, S., Filiano, J., Karnes, P.S., Patterson, M.C., & Freeze H.H. (2000) Dolichol phosphate mannose synthase (DPM1) mutations define congenital disorders of glycosylation (Ie). ***Journal of Clinical Investigation***. 105:191-198. PMCID:PMC377427
6. Westphal, V., Murch, S., Kim, S., **Srikrishna, G.**, Winchester, B., Day, R., & Freeze, H.H. (2000) Reduced heparan sulfate accumulation in enterocyte contributes to protein-losing enteropathy in Congenital Disorder of Glycosylation (CDG-Ic). ***American Journal of Pathology*** 157:1917-1925. PMCID: PMC1885788
7. Westphal,V., **Srikrishna, G.**, & Freeze,H.H (2000) Congenital disorders of glycosylation: Have you encountered them? ***Genetics in Medicine*** (Review) 2:329-337. PMID: 11339653
8. Davis, JA, Wu, X-H, Wang, L., DeRossi, C., Westphal, V., Wu, R., Alton, G., **Srikrishna G.**, and Freeze HH. (2002). Molecular cloning, gene organization and expression of mouse *Mpi* encoding phosphomannose isomerase ***Glycobiology*** 12, 435-442. PMID:12122025

Publications from Christian Medical College Hospital, Vellore, India

1. **Srikrishna, G.**, Mathai, D., Abraham, O.C., Kaur, A., Kanagasabapathy, A.S., and Pulimood, B.M (1994). IgG multiple myeloma and alloalbuminemia: an unusual association. ***Journal of Association of Physicians of India***, 42: 331-332
2. Swaminathan, S., **Srikrishna, G.**, Seshadri, M.S., Punnoose, M., and Kanagasabapathy, A.S (1993). Detection and elimination of preanalytical errors in the determination of zinc in biological samples. ***Indian Journal of Medical Research***, 98: 199-201.
3. Swaminathan, S., **Srikrishna, G.**, Selvakumar, R., Seshadri, M.S., and Kanagasabapathy, A.S (1993). Ethanediol stabilized quality control serum for measurement of zinc. ***Clinica Chimica Acta***, 215: 119-120.
4. Kuruvilla, A., **Srikrishna, G.**, Peedicayil, J. Kuruvilla, K., and Kanagasabapathy, A.S (1993). A study of serum prolaction levels in schizophrenia: correlation with positive and negative symptoms. ***International Journal of Clinical Pscychopharmacology***, 8: 177-179.
5. Kuruvilla, A., **Srikrishna, G.**, Peedicayil, J. Kuruvilla, K., and Kanagasabapathy, A.S (1992). A study of serum prolaction levels in schizophrenics: comparison of males and females. ***Clinical and Experimental Pharmacology and Physiology***, 19: 603-606.
6. Kuruvilla, A., **Srikrishna, G.**, Peedicayil, J., Kuruvilla, K., and Kanagasabapathy, A.S (1991). Serum prolactin levels in mania. ***Biological Psychiatry***, 30: 421-423.
7. **Srikrishna, G.**, and Kanagasabapathy, A.S. (1989). A peptidase activity from primate liver that inactivates oxytocin in vitro: purification and partial characterization. ***Journal of Endocrinology***, 121: 537-544.
8. **Gopalaswamy,G*.**, Srikrishna, K., and Kanagasabapathy (1984). Staining of cystyl aminopeptidase ('oxytocinase') isoenzymes on polyacrylamide gels. ***Clinical Chemistry***, 30, 1115-1116
9. **Gopalaswamy,G*.**, Balasubramaniam, N., and Kanagasabapathy, A.S (1984). Fractionation of cystine aminopeptidase ('oxytocinase') from term human placenta and maternal serum. ***Clinica Chimica Acta*** 144; 39-48
10. **Gopalaswamy,G*.**, Balasubramaniam,N.,and Kanagasabapthy, A.S. (1983). Cysylaminopeptidase in maternal serum for the antenatal recognition of fetal growth retardation. ***Australian and New Zealand Journal of Obstetrics and Gynecology***, 23, 79-84.

* Maiden name